*BIOSYNTHESIS OF β-D-GALACTOSIDASE CONTROLLED BY PHAGE-CARRIED GENES. I. INDUCED β-D-GALACTOSIDASE BIOSYNTHESIS AFTER TRANSDUCTION OF GENE z⁺ BY PHAGE**

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Transduction of the *lac*⁺ character to *lac*⁻ strains of the *Escherichia coli–Shigella dysenteriae* group of organisms can be performed by lysates of phage P1 released by *lac*⁺ bacterial donors.[1, 2] The production of high-frequency transducing (HFT) lysates has been reported.[3, 4] The transducing activity in these lysates is embodied in defective phage particles P1*dl*, which carry the entire *lac* genetic region of the donor bacterium. The relations between P1 and P1*dl* and between these phages and the host bacteria resemble in many (although not in all) respects the relations between phage λ, its *gal* transducing derivatives λ*dg*, and their bacterial hosts.[5, 6]

The well-known properties of the cellular components—β-D-galactosidase,[7] galactoside-permease,[8] and acetylase[9]—controlled by the *lac* genes, and the remarkable body of information available concerning the structure, organization, and functional regulation of these genes,[10] make it desirable to study the function of these genes when they are part of phage-related elements, which can assume various types of relation to their bacterial host, and to compare it to the function of the same genes in chromosomal structures, that can be transferred by mating.[11, 12] The present paper describes the initiation of function of a phage-associated *z*⁺ gene—the genetic determinant of galactosidase—upon entering a bacterial cell. The following papers[13, 14] report some interactions between phage associated genes, their chromosomal counterparts, and the phage-immunity control system.

Materials and Methods.—Many of the strains, media, and procedures have been described previously.[3, 4] Table 1 summarizes the relevant properties of the bacterial strains used. The properties of the *lac* genes are classified as states of the four genes: *i* (repressor), *o* (operator),

TABLE 1

PROPERTIES OF BACTERIAL STRAINS

Strain	Properties*
E. coli K-12 3.000	$i^+o^+z^+y^+$
" " 3.300	$i^-o^+z^+y^+$
" " 3.320	$i^-o^cz^+y^+$
" " 2.000 o^c	$i^+o^cz^+y^+$
" " 2.0S0, 3.0S0, 3.0U0	$i^+o^+z^-y^+$
" " 2.0S0R	$i^+o^+z^+y^+$ (revertant from 2.0S0)
" " W4032(λ)	lac^{del}
" " W4032 = W4032λ^s	From W4032(λ); λ-cured by UV
" " W4032 *lac*⁺	Stable *lac*⁺ transductant from W4032
" " W4032-W-1, 2.0S0-W-1	Strains carrying prophage P1*dl* $i^+o^+z^+y^+$
" " W4032-W-5	Strain carrying prophages P1 and P1*dlw* $i^+o^+z^+y^+$
" " W4032-13-4, 2.0S0-13-4, 3.0S0-13-4-1, 3.0S0-13-4-2	Strains carrying prophage P1*dl* $i^-o^+z^+y^+$
S. dysenteriae Sh	$i^+o^+z^-y^{del}$
" " Sh I-4	Sh carrying prophage P1*dl* $i^+o^+z^+y^+$
" " Sh 31-20	Sh carrying P1*dlw* $i^+o^+z^+y^+$
" " Sh 125-13-4	Sh carrying P1*dl* $i^-o^+z^+y^+$

* More complete information on the properties and sources of these strains is given elsewhere.[3, 4] P1-lysogenic derivatives were prepared as needed.

z (galactosidase), and y (permease-acetylase) according to the scheme of Jacob and Monod.[10] The i^- strains are normal constitutives; o^c strains are constitutive dominants; o^0 strains are operator negative.

The lac^+ HFT lysates were all derived from strain P1kc of phage[1] (here called P1 for brevity's sake) and contained mixtures of normal P1 and of transducing P1dl particles; the transducing particles are classified as P1dl i^+z^+, P1dl i^-z^+, etc., according to the properties of the lac genes they carry; these correspond to the properties of the lac^+ donor from which the transducing phage were derived. P1dlw i^+z^+ is a variety of P1dl phage used in many experiments.[3] Low-frequency-transducing (LFT) lysates were lysates of phage P1 grown on normal lac^+ bacteria.[3]

The HFT lysates were produced by collecting bacteria from a growing culture, treating them with ultraviolet (UV) light (40 sec at 50 cm from a GE "germicidal" bulb), superinfecting with active P1 at a multiplicity of infection (m.o.i.) of about 2, and incubating 2.5 hr in LB broth at 37° before centrifuging and filtering through Millipore membranes. The phage was concentrated and freed of any galactosidase by centrifuging twice at 16,000 × g for 1 hr. The phage pellet was resuspended in TGA medium minus glycerol, supplemented with 0.1 per cent gelatin.

TGA medium (NaCl, 0.08 M; KCl, 0.02 M; NH_4Cl, 0.02 M; $MgCl_2$, 10^{-3} M; $CaCl_2$, 2×10^{-4} M; $FeCl_3$, 2×10^{-6} M; Na_2SO_4, 2.5×10^{-3} M; Tris (hydroxymethyl) aminomethane, 0.12 M; K_2HPO_4, 2×10^{-4} M; pH 7.5, supplemented with 0.2 per cent glycerol, 0.5 μg/ml thiamine, and 0.1 per cent tryptone or 0.1 per cent casein hydrolysate) was the growth medium. The doubling time for *E. coli* strains in this medium is 56 to 60 min at 37°C. $CaCl_2$ was added at a concentration of 2.5×10^{-3} M for adsorption of phage.

Reducing buffer for galactosidase assay: sodium phosphate, 10^{-1} M; $MgSO_4$, 10^{-3} M; $MnSO_4$, 2×10^{-4} M; mercaptoethanol, 10^{-1} M; pH 7.0.

Optical density (O.D.) of experimental cultures was read at 500 mμ in a Zeiss spectrophotometer. For *E. coli* bacteria in TGA medium, an O.D. of 0.18 corresponds to 1×10^8 cells/ml.

Transductions were assayed by plating suitable dilutions of phage-bacterial mixtures on EMB lactose agar plates. An excess of lac^- bacteria was added to provide a uniform background.

For galactosidase assay, the o-nitrophenyl-β-D-galactoside (ONPG) method[11, 15, 16] was used. One-ml aliquots of a growing culture ($<4 \times 10^8$ bacteria/ml) were added to 1-ml samples of reducing buffer containing 0.01 per cent sodium desoxycholate and 0.02 ml toluene. The tubes were stoppered, shaken vigorously, and incubated with shaking at 37° for 30 min. After equilibration at 28°C, 0.6 ml of M/75 ONPG in M/4 phosphate buffer (pH 7.0) was added and the tubes were incubated until a visible yellow color had developed. The reaction was stopped by addition of 1.3 ml 1 M sodium carbonate and the optical density was measured at 420 mμ in a Zeiss spectrophotometer. A correction for turbidity at 550 mμ was made.[11] One unit of enzyme is defined as the amount of enzyme that releases 1 mμmole of *o*-nitrophenol (= ONP) per minute at 28°, pH 7.0; a solution of 1 mμmole of ONP per ml has an optical density of 0.005 under the above conditions (final pH = 10.2; 10 mm light path).

The inducers for galactosidase were methyl-thio-β-D-galactoside (TMG) or isopropyl-thio-β-D-galactoside (IPTG). ONPG, TMG, and IPTG were products of Mann Biochemical Co. (The initial sample of IPTG was kindly provided by Dr. J. Monod.)

The studies with the fluorogenic substrate 6-hydroxyfluoran-β-D-galactoside (6HFG) were done by the method of Rotman.[17]

Results.—Production of β-galactosidase after introduction of P1dl z^+ into z^- bacteria in the presence of inducer: A typical experiment consists of mixing a transducing phage preparation with log-phase z^- bacteria at about 2×10^8 per ml in TGA medium plus $CaCl_2$ at 37°C, allowing 2 to 15 min for phage adsorption, and diluting 1:10 in prewarmed medium (without added $CaCl_2$) containing 10^{-3} M TMG or 2×10^{-4} M IPTG as inducers. Incubation is continued in a shaker bath at 37°C using shallow layers of culture in Erlenmeyer flasks. Samples for assays of total bacteria, of lac^+ transductants, and of galactosidase are taken at intervals. Free phage was also measured in some experiments. The results of an experiment of this kind, in which the recipient bacteria were *E. coli* i^+z^-, are shown in Figure 1. In this experiment, the ratio of P1-plaque formers to bacteria was 0.2. It is seen that

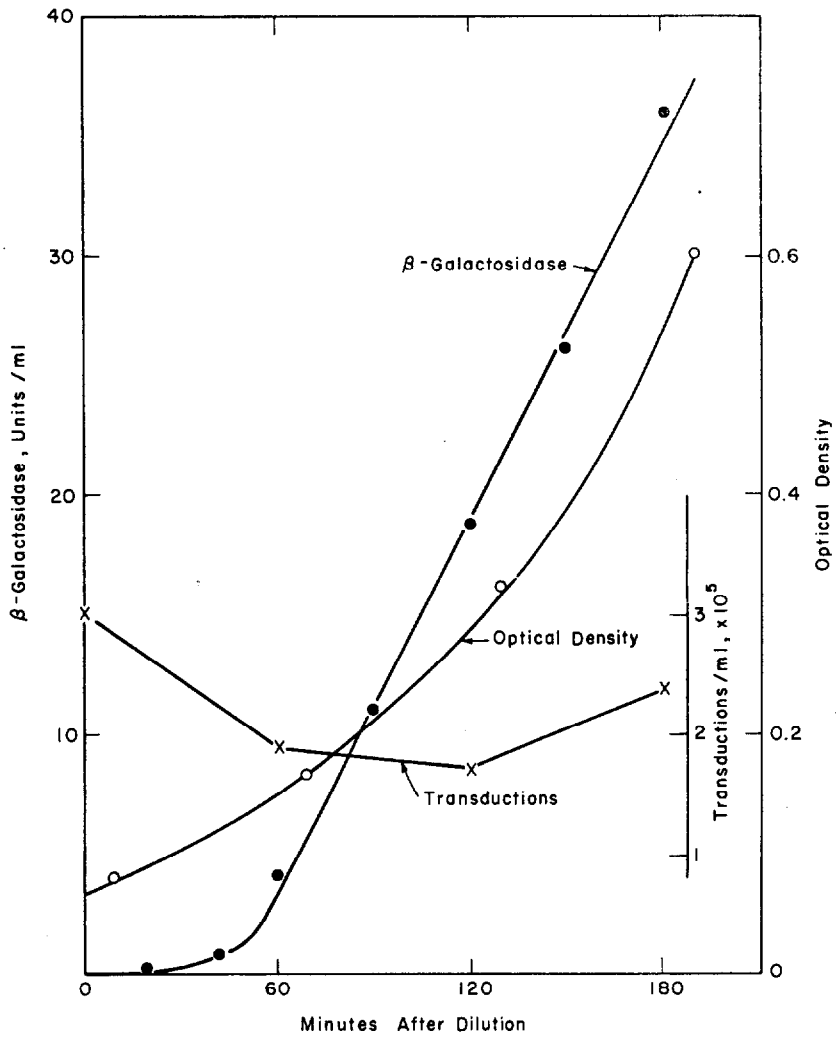

FIG. 1.—Bacterial growth, *lac*$^+$ transductions, and galactosidase synthesis after infection of *E. coli* 2.0S0 i^+z^- bacteria with an HFT lysate containing P1*dlw* i^+z^+. The cell-phage mixture, after incubation in TGA medium + $CaCl_2$ for 15 min., was diluted at time 0 in TGA medium with 10^{-3} *M* TMG.

the bacterial population as a whole grew exponentially, while the transductions, scored as *lac*$^+$ colonies, remained more or less constant for at least 3 hr. Galactos idase was present from the time of the earliest measurement (20 min) and increased at first at an accelerated rate, then linearly for about 3 hr.

Essentially similar results were obtained in experiments of this type (*in the presence of an inducer*) whether the inducer was added at dilution time, as in Figure 1, or at infection time; whether the adsorption period was 2 or 15 min; whether the transduced genes were i^-z^+, i^+z^+, or $i^+o^cz^+$; and whether the recipient *lac*$^-$ bacteria were *E. coli* i^+z^-, i^-z^-, $i^-z^-y^-$ ($=lac^{del}$), or i^-o^o. Quantitative differences in the rate of enzyme production reflect differences in phage-adsorption rate among different bacterial strains (Fig. 2).

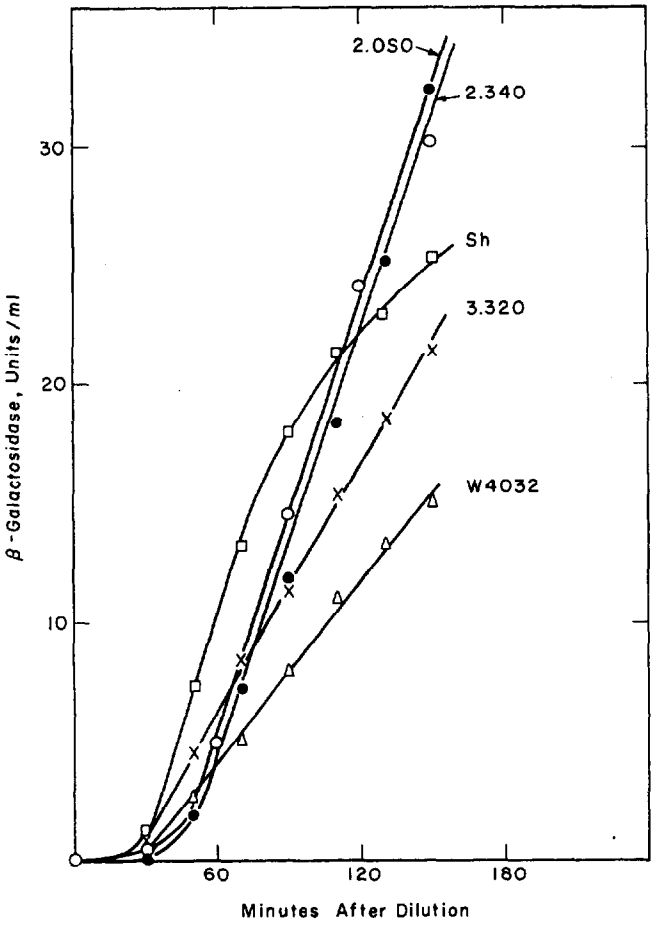

FIG. 2.—Production of galactosidase after infection of different *lac*$^-$ recipient strains with a lysate of P1*dlw* i^+z^+ in the presence of 10^{-3} *M* TMG. The adsorption of phage P1, measured for each strain under the conditions of this experiment, was as follows: strains 2.0S0, 2.340, and 3.320: 50–60%; strain W4032; 20–30%; strain Sh: 75–85%.

When the recipient is *S. dysenteriae* Sh there is usually a faster rate of enzyme production in the linear phase, due to better phage adsorption, and a drop in enzyme production rate at about the time of lysis (see Fig. 2), probably correlated with death of some of the bacteria.

The results of this experiment are the same if the adsorption mixture, instead of being diluted to stop phage adsorption, is centrifuged at room temperature and the bacteria are resuspended in calcium-free medium with inducer. With all recipients, only a very small fraction, from 1 to 7 per cent, of the galactosidase formed after infection is released into the medium; this release is fully accounted for by the minority of bacteria that are lysed due to infection with active phage P1.

Two aspects of enzyme production require explanation: the initial phase of acceleration and the later prolonged phase of linear synthesis.

Phase of accelerated synthesis: Except for minor differences, this phase is present in all combinations of phage and recipient types. It is not due to a delayed effect

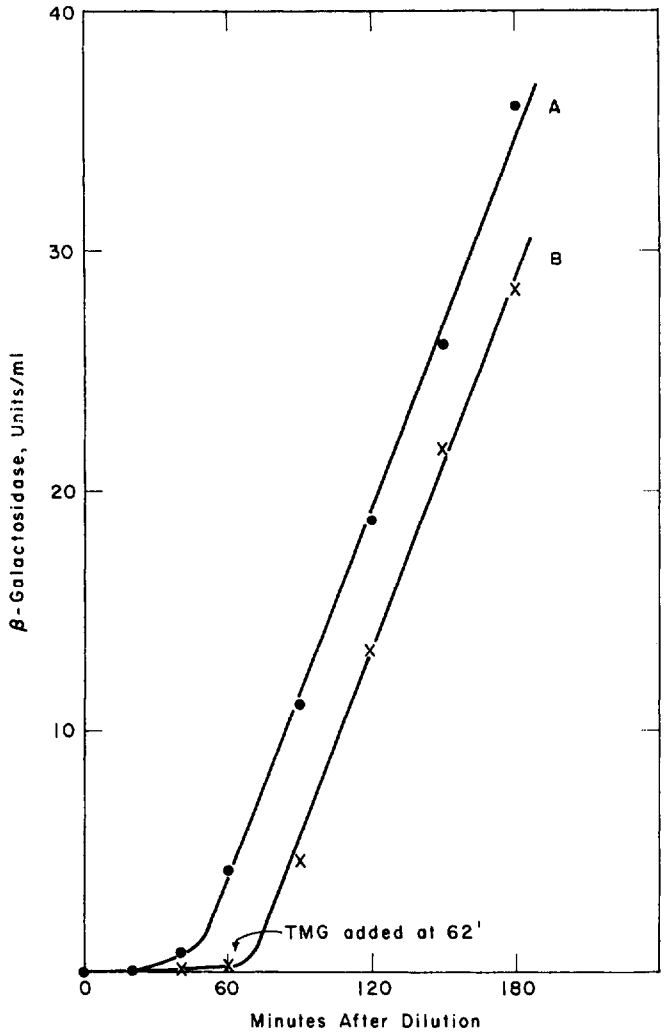

FIG. 3.—Dissociation of inducer function from the acceleration phase of enzyme synthesis. Phage P1*dlw* i^+z^+ on *E. coli* 2.0S0 i^+z^- recipient. *A*, TMG added at time 0, as in Figure 1. *B*, TMG added at 62 min.

of inducer, because if the inducer is added at 60 or 90 min after infection the linear phase begins almost immediately, as shown in Figure 3. The 3- to 5-min delay corresponds to the delay observed[18] for initiation of response to inducer. The steps needed to attain the final linear rate of enzyme production do not require protein synthesis: if infection is done in the presence of 5-methyltryptophan, which stops protein synthesis completely, a later addition of tryptophan causes an almost immediate start of linear enzyme production (Fig. 4). Similar experiments with chloramphenicol instead of 5-methyltryptophan gave ambiguous results, probably because of slow recovery of protein synthesis after removal of the drug by washing. Mitomycin treatment gave rapid loss of enzyme-synthesizing capacity; other inhibitors of DNA or RNA synthesis have not been tested.

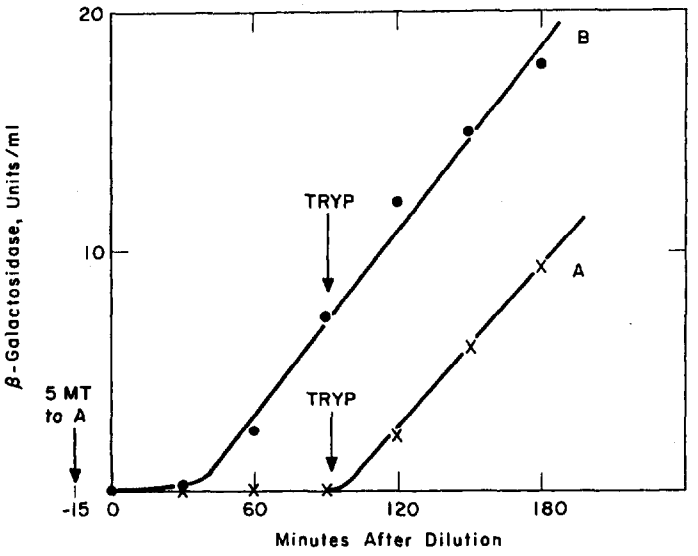

FIG. 4.—The effect of 5-methyltryptophan on the early enzyme synthesis. Lysate P1*dlw* i^+z^+ on *E. coli* 2.340 i^-z^- recipient. At the time of mixing bacteria with phage, culture *A* received 100 μg/ml DL-5-methyltryptophan. L-tryptophan, 100 μg/ml, was added to both cultures *A* and *B* (control) at 92 min. TMG was present from time 0 in both cultures. The number of stable *lac*$^+$ transductions was the same in both cultures.

The accelerated phase reflects either a delay or a heterogeneity in the accomplishment of some early reactions; these may be some of the early steps in phage-cell interaction. The latent period for growth of phage P1 at 37°C in TGA medium is about 60 min; but no information is available as to the early synthesis of P1-phage materials. An attempt to suppress enzyme production by treatment in a Lourdes homogenizer (blendor technique) at 5 min after infection gave negative results, which, therefore, neither support nor exclude the possibility of delayed injection of genetic material by phage particles. The hypothesis of a multiplication of the transduced genetic elements will be discussed later.

Phase of linear synthesis: The most immediate question concerns the relation of linear galactosidase synthesis to the cells that give rise to *lac*$^+$ transductant colonies. On the one hand, linear synthesis suggests either a constant rate of function of nonmultiplying genetic elements (abortive transduction[19, 20]), or some other limitations in the amount of enzyme that can be made per unit time. On the other hand, the roughly constant number of *lac*$^+$ colonies in platings done over a period of several hours (see Fig. 1) indicates either a nonmultiplying group of cells or the segregation of *lac*$^-$ cells from a steady number of *lac*$^+$ bacteria.[21] Segregation with occasional late integration is suggested by the finding that, when few infected bacteria are plated on EMB lactose agar so that isolated colonies can be observed, most of the *lac*$^+$ transductants appear late, as small papillae within well developed *lac*$^-$ colonies.

A quantitative relation between number of transductions and amount of enzyme can be obtained from experiments such as that of Figure 1. In that experiment, for example, the amount of enzyme synthesized in 60 min (corresponding to one doubling time) during the linear phase is about 15 units, corresponding to the pro-

duction of 6×10^6 fully induced *lac*$^+$ cells. This is twenty times higher than the number of *lac*$^+$ transductants measured at 0 time. A series of results, with different combinations of lysates and recipient strains and with different ratios of lysate to cells, is given in Table 2. The excess of enzyme over transductions is present in all cases. Inspection of Table 2 reveals that there is no correlation between

TABLE 2

RATES OF LINEAR SYNTHESIS OF GALACTOSIDASE AND NUMBERS OF STABLE TRANSDUCTIONS FOR VARIOUS DONOR-RECIPIENT COMBINATIONS*

Expt. no.	HFT lysate type	Recipient strain	Normal P1 helper added	M.o.i. P1	Transductions per ml	Enzyme units/ml/hour	Excess enzyme†
29	W-5:	*E. coli* i^+z^-	−	0.05	8.3×10^4	2.6	13
"	P1*dlw* i^+z^+	" "	+	~1.5	6.3×10^4	1.3	8.3
"		" *lac*^{del}	−	0.05	1.4×10^3	0.8	228
"		" "	+	~1.0	1.8×10^4	0.8	18
"		*Shigella* i^+z^-	−	0.05	1.0×10^4	3.7	148
"		" "	+	~2.0	1.3×10^5	1.8	14
36		*E. coli* i^-z^-	−	0.1	3.4×10^4	2.7	32
"		*Shigella* i^+z^-	−	0.1	2.0×10^4	8.4	164
45	W-1:	*E. coli* i^+z^-	−	0.2	3.7×10^5	4.5	4.8
"	P1*dl* i^+z^+	" i^-z^-	−	0.1	1.3×10^5	1.1	3.4
"		*Shigella* i^+z^-	−	0.4	6.2×10^4	4.2	27
69	13-4:	*E. coli* i^+z^-	−	<0.1	1.1×10^5	3.8	14
"	P1*dl* i^-z^+	" i^-z^-	−	<0.1	6.8×10^4	6.0	35
92		" i^+z^-	−	~0.25	1.2×10^5	6.8	23
"		*Shigella* i^+z^-	−	~0.25	1.0×10^4	5.5	220
53	P1*dl*	*E. coli* i^+z^-	−	0.13	6.7×10^4	0.6	3.6
"	$i^+o^cz^+$	" *lac*^{del}	−	0.11	4.8×10^3	0.6	48
"		*Shigella* i^+z^-	−	0.15	1.9×10^4	1.3	28

* *Lac*$^+$ transductions were measured between 0 and 30 min after the end of phage adsorption. The amounts of enzyme are those formed in 1 hr during the linear phase of synthesis (see Fig. 1). Both values are normalized to 1 ml of infected cell suspension.

† The "excess enzyme" values are the ratios of the amounts of enzyme produced per hour (normalized to the amount of enzyme contained in one fully induced z^+ cell $\simeq 2.5 \times 10^{-6}$ units) to the number of *lac*$^+$ transductions. Note the large excess values in transductions to recipients like *Shigella* or *E. coli lac*^{del}, which require helper phage to give stable transductions.

numbers of *lac*$^+$ transductants and amounts of enzyme. This is particularly evident when one compares *E. coli* z^- recipients, in which transduction is frequent and occurs by integration of the transduced genes into the recipient chromosome, with recipients such as *S. dysenteriae* or *E. coli lac*^{del}, in which transduction is rare and occurs by lysogenization, with a requirement for active phage P1 as helper.[3] The enzyme levels are roughly similar with all recipients, as though similar numbers of cells received the *lac*$^+$ genes and made enzyme, but different proportions of them gave *lac*$^+$ transductant colonies.

The hypothesis that the excess of enzyme over transduction is due to abortive transductions, that is, to the presence of bacteria that have received P1*dl* phage particles whose genes can function but do not become established, appears to account for all the above findings. An alternative explanation would be that only those bacterial cells that will give complete transductants are making enzyme, and that the enzyme levels are exceptionally high because the incoming *lac*$^+$ element, being partly phage, multiplies vegetatively producing many copies of the *lac*$^+$ genes per cell. This explanation is hard to entertain, because if the amounts of galactosidase observed in the experiments of Table 2 were made in the relatively few *lac*$^+$ transductant cells this would require an enormous increase in cellular

protein; the galactosidase in fully induced normal *E. coli* cells already represents over 5 per cent of the total protein.[7]

Yet, the hypothesis that multiplication of the lac^+ genes is responsible for the enzyme excess must be entertained seriously because of another set of observations, illustrated in Figure 5. This refers to transduction of P1-lysogenic recipients and

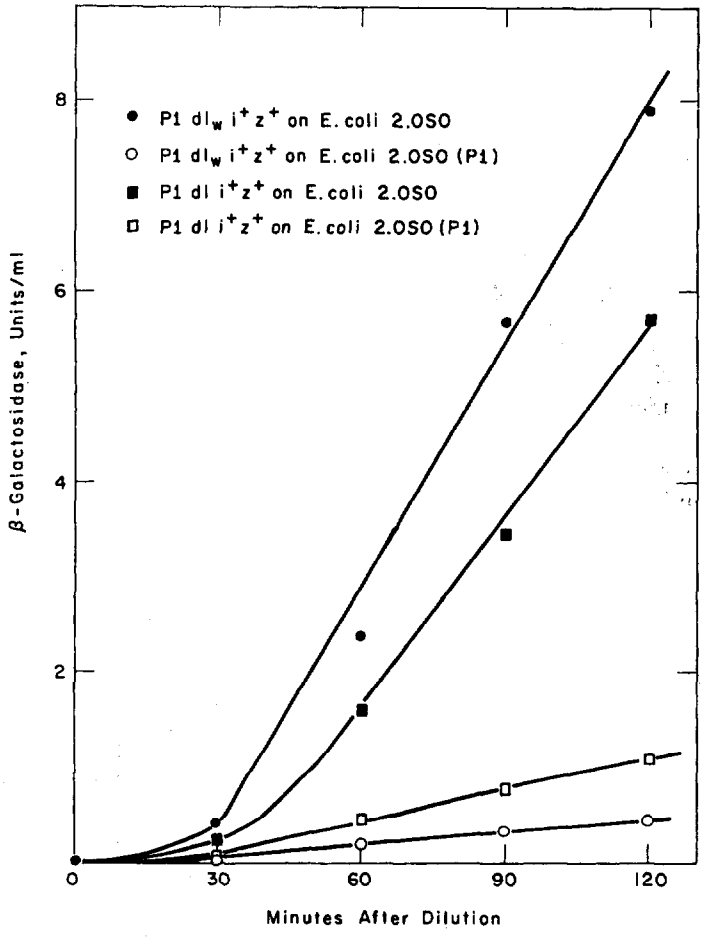

FIG. 5.—Synthesis of galactosidase in P1-sensitive and P1-lysogenic recipients. The donor phages were P1*dlw* and P1*dl*, both i^+z^+. The recipient bacteria were *E. coli* 2.0S0 and 2.0S0(P1), both i^+z^-. The rates of adsorption of P1 to these two strains are identical.

will be discussed more fully in the following paper. The rate of enzyme production with lysogenic recipients is lower than with P1-sensitive ones, the ratio varying with the type of phage lysate and the nature of the recipient strain from 1:2.3 to 1:67 (see ref. 13, Table 3). It is known[22] that, at least with some of the temperate phages, a phage that superinfects a bacterium already lysogenic for a related coimmune prophage fails to multiply vegetatively. Thus, the lower enzyme levels observed with lysogenic recipients might reflect repressed multiplication of the transducing phage elements.

The number of bacteria with galactosidase after transduction: To settle this question, enzyme studies on individual cells were done using a modification of a recently devised fluorogenic assay.[17] This consists in observing microscopically the fluorescence of 6-hydroxy-fluoran (6HF) released by the action of galactosidase on the nonfluorescent substrate 6HFG. A mixture of bacteria and substrate in a suitable buffer is prepared and dispersed in microdrops under silicone oil by spraying a fine mist of suspension. Alternatively, bacteria are distributed into microdrops by means of a micromanipulator. The fluorescence developed upon incubation in single drops of appropriate size is observed microscopically with appropriate light filters or can be measured with a photomultiplier attachment. The number of bacteria present in a microdrop can be counted with dark field illumination.

Control experiments showed that when intact, fully induced *lac*$^+$ cells are used, only about 10 per cent of them give a positive reaction; but a brief treatment with toluene (5 min at room temperature) increases the proportion of positive reactions to between 50 and 100 per cent, without releasing enough free enzyme to give any diffuse background fluorescence.

Accordingly, a series of experiments similar to that shown in Figure 1 were done, in which, in addition to the other measurements, a sample was taken near the 100th minute after infection and used for a count of the number of cells with enzyme. The 100-min time was chosen because by then the linear rate of synthesis is established, a substantial amount of enzyme has been synthesized, and yet the recipient cells have multiplied at most by a factor 3.2 (1.7 generations). The results are shown in Table 3. It is clear that in every experiment there was a substantial

TABLE 3

THE NUMBERS OF CELLS WITH GALACTOSIDASE AT 100 MINUTES AFTER INFECTION

Experiment	Recipient strain	$\frac{\text{Transductions}}{\text{Total cells}} = a$	$\frac{\text{Cells with enzyme}}{\text{Total cells}} = b$	$b:a$
6-27-2	*E. coli* i^+z^-	4.0×10^{-3}	2×10^{-2}	5
6-27-3	" i^-z^-	2.0×10^{-3}	2×10^{-2}	10
6-28-5	" i^-z^-	2.8×10^{-3}	$2\text{–}3 \times 10^{-2}$	7–10
6-29-6	" i^+z^-	6.0×10^{-4}	1×10^{-2}	16
6-29-6	" i^-z^-	6.0×10^{-4}	8×10^{-3}	13
6-30-7	" i^+z^-	3.6×10^{-3}	$3\text{–}4 \times 10^{-2}$	8–11
6-30-7	" i^-z^-	1.6×10^{-3}	(8×10^{-2})	(50)
7-1-9	" i^-z^-	$2\text{–}4.0 \times 10^{-3}$	2×10^{-2}	5–10

HFT lysates containing P1*dl* i^+z^+ were used in all experiments. The values in parentheses are based on a poor count and may be too high.

excess, from 5- to 16-fold, of cells with enzyme over *lac*$^+$ transductants. Even if each of the potential transductants had given rise on the average to three cells with enzyme (of which only one would ultimately give a *lac*$^+$ clone since the number of *lac*$^+$ colonies does not increase for 4 hr or longer) this could not account for the number of cells with enzyme found at 100 min. In fact, since the scoring is less than 100 per cent effective, the excess of positive cells over a complete transductions is probably even greater.

We conclude that most of the enzyme produced during the phase of linear synthesis is made in cells that do not register as *lac*$^+$ transductants in platings because they do not give rise to clones of *lac*$^+$ cells, at least under the conditions of our experiments. These are the equivalent of abortive transductants.

Ultraviolet irradiation of HFT transducing lysates: Small doses of UV irradiation

increase the number of complete transductants for the *lac*$^+$ marker[3] as well as for other genetic markers,[23, 24] while reducing the number of abortive transductions. Several experiments with UV-treated lysates were done in an attempt to study the inactivation of the genetic element responsible for galactosidase production in newly infected z^- bacteria. These experiments did not fulfill the expected goal. It was found that the apparent rate of inactivation of phage P1, measured by preadsorption and plating on sensitive bacteria, varied greatly with the bacterial host strain. For a given strain, the plaque-forming ability of phage P1 and the galactosidase-forming capacity of the infected cells (measured from the rate of enzyme production in the linear phase) were closely parallel. This result suggests that some UV-sensitive, host-specific key function of the phage is needed to initiate both infection by active P1 and galactosidase production by P1*dl*. The previously reported results[3] on the effect of UV on the frequency of transduction by P1*dl* on various hosts lose much of their potential significance since they reflect mainly the UV-sensitivity of the host-specific phage functions.

Transduction by LFT lysates: The study of galactosidase production after infection with LFT lysates is hampered by their low transducing activity, because measurable enzyme levels are obtained only with concentrations of lysate that give high multiple P1 infection. This in turn depresses enzyme synthesis, probably through damage to cells and early lysis. Using an LFT lysate with an unusually high transducing titer (3.8×10^5 *lac*$^+$ colonies for 2.2×10^9 active P1 per ml), it was found that the amount of galactosidase produced was again four-fold in excess over that corresponding to the *lac*$^+$ transductants. Hence, with LFT as well as with HFT lysates, there appears to be extensive abortive transduction.

Discussion.—Abortive transduction was recognized originally with genes controlling bacterial flagellation, by the occurrence of motile cells that gave rise to clones consisting of nonmotile descendants plus one line of motile cells (unilinear transmission of the motility trait).[19] Later, abortive transduction was invoked in transduction of nutritional markers to explain the appearance of unsupplemented media of small colonies containing large numbers of genetically auxotrophic bacteria plus one genetically autotrophic cell.[20] The enzyme produced by a single autotrophic cell in each generation appeared to be sufficient, when distributed among the descendant cells, to supply the synthetic requirements for several generations of growth. The *lac* case resembles the nutritional mutant situation in that the gene studied controls a well-defined enzymatic reaction, and the motility situation in that the function of the transduced gene can be recognized directly on individual cells.

The linear rate of production of galactosidase can be explained by the scheme of Figure 6, in which it is assumed that enzyme is produced, at a constant rate per unit time, only in those cells that have the (nonmultiplying) z^+ gene. Since galactosidase is very stable under the conditions of these experiments, measurements of the actual amounts of enzyme in individual cells at various times after transduction, using the fluorogenic method,[17] should make it possible to check both the distribution function of enzyme at cellular division and the actual rates of enzyme synthesis per cell early after transduction. These in turn may reveal whether any multiplication of the incoming genes occurs during the early phase of accelerated enzyme synthesis.

The occurrence of abortive transduction shows that enzyme production after transduction is not correlated with integration of the newly entered genes into the recipient chromosome. This situation differs from the transfer of the *mal*$^+$ genes as free DNA in transformation of pneumococci,[25] where the early rate of enzyme synthesis corresponds closely to the number of bacteria in which integration occurs and which yield stably-transformed clones. This finding may reflect the remarkable speed of integration of genes transferred as free DNA[26] rather than an inability

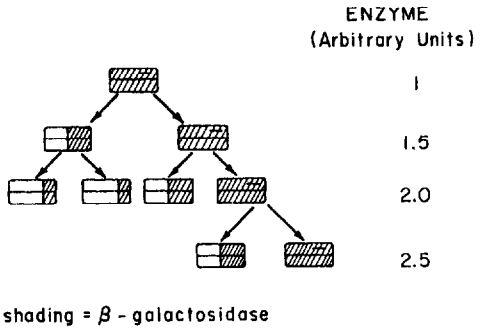

FIG. 6.—Linear inheritance of nonmultiplying z^+ gene.

of such genes to function from a nonchromosomal location. It is not excluded, however, that the presence in transducing particles of some phage genes is required for the autonomous function of the transduced genes. The early accelerated phase of enzyme production after transduction may reflect the need for a sequential initiation of the function of such phage genes. The biosynthesis of enzymes controlled by transduced genes may resemble in this respect the biosynthesis of enzymes and other proteins controlled by phage genes, which also exhibits a characteristic sequential progression.[27]

It is interesting to compare the course of galactosidase production after transduction with that after mating *lac*$^+$ male bacteria with *lac*$^-$ females.[11, 12] In mating, the z^+ gene from the donor appears to function immediately at maximum rate after entering the recipient cell, without an early acceleration phase. This again suggests that the phase of acceleration observed early after transduction reflects some specific requirements of genes entering a part of a phage.

The only other system of high-frequency transduction available, the λ-λ*dg* system, provides an opportunity to study the synthesis of the galactose-utilizing enzymes after introduction of the corresponding genes by phage into *gal*$^-$ recipient cells. Preliminary reports[28] indicate that the course of synthesis resembles that of galactosidase described here; but the shortcomings of assay methods for these enzymes, more cumbersome and less sensitive than those for galactosidase, have apparently hampered progress of this work. Actually, the P1*dl* transduction system, because of a felicitous combination of circumstances, may offer a unique material for a variety of studies on enzyme biosynthesis. For example, P^{32}-labeled phage P1*dl* can be used to study the effects of damage in the DNA of a gene at various stages of its functional career. Also, P1*dl* phage provides a source of a DNA specialized in the control of an easily measurable enzyme for use in study of enzyme synthesis in cell-free preparations. Experiments along these lines are

in progress. The following papers[13, 14] report studies on the role of repression in the function of *lac* genes in phage P1*dl*.

Summary.—Upon introduction of the *lac*$^+$ genetic determinants of *E. coli* into *lac*$^-$ bacterial cells by transducing particles derived from phage P1, synthesis of β-D-galactosidase occurs, first at an accelerated rate, then at a linear rate for a period of several hours. The phase of accelerated synthesis is attributed to the initiation of function of phage genes in the transducing particles; the phase of linear synthesis, to the production of enzyme under control of nonmultiplying *lac*$^+$ genes. Enzyme is produced by many cells which, although they have received a functional transducing particle, fail to give rise to permanently transduced cell lines (abortive transduction). The counting of individual bacterial cells containing β-D-galactosidase in a mixed population is described.

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